Supplementary Information

Etv4 and Etv5 are required downstream of GDNF and Ret for kidney branching morphogenesis.

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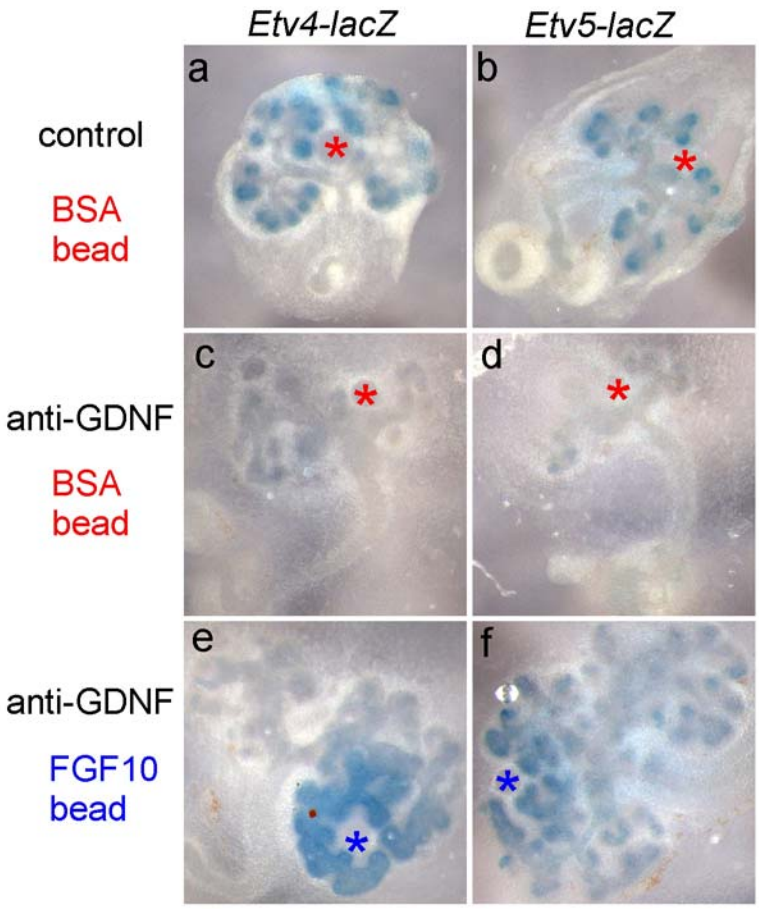
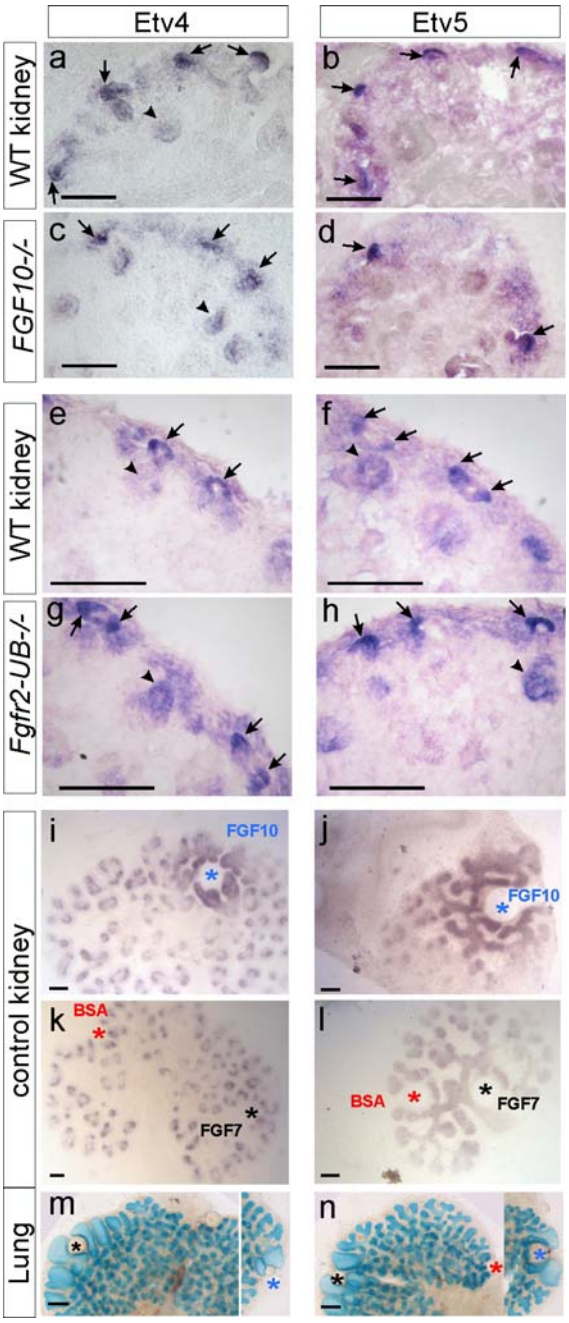
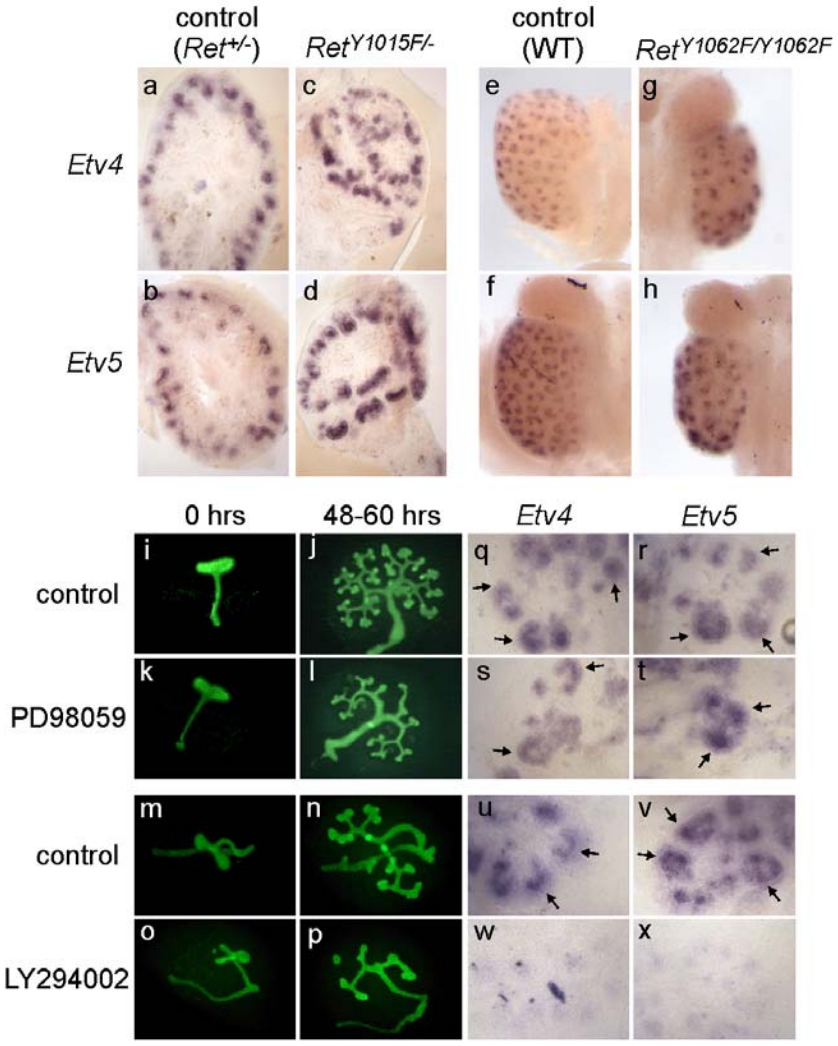


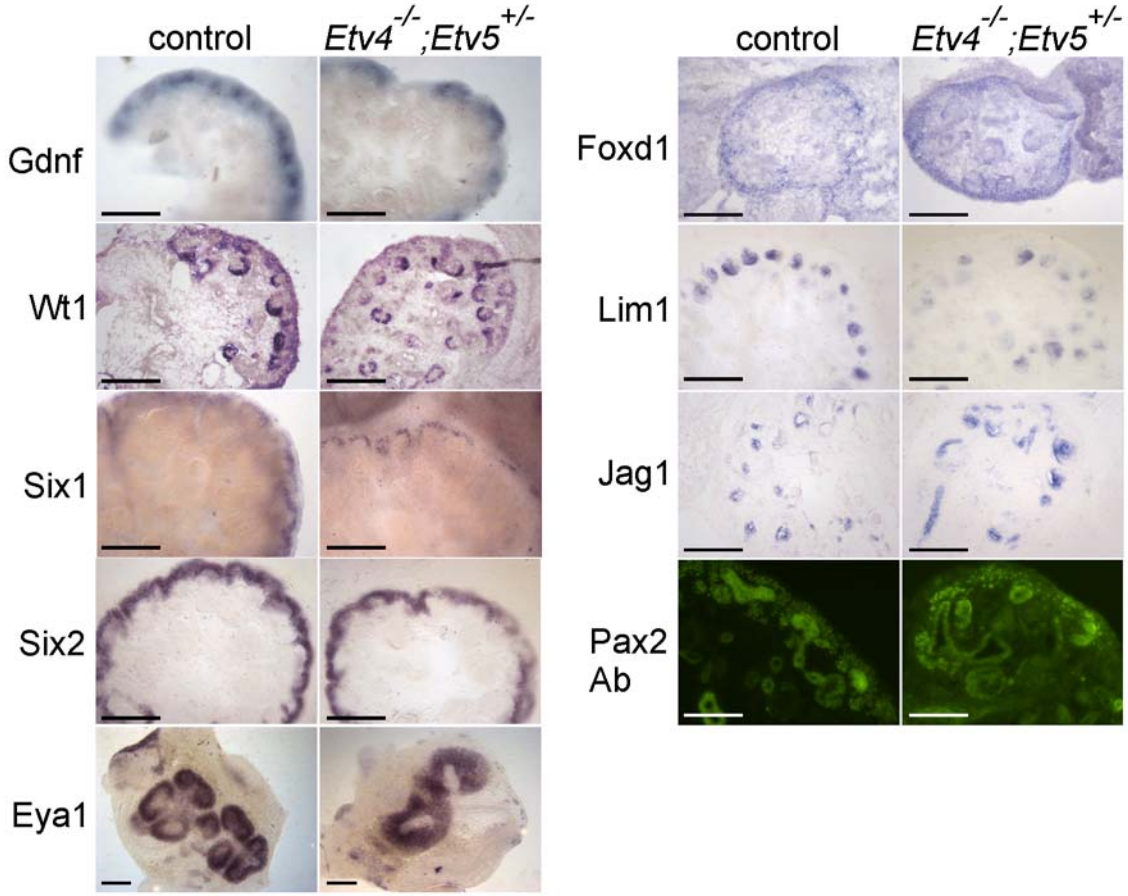
Supplementary Figure 1. Expression of *Etv4* and *Etv5* is blocked by anti-GDNF, and can be restored by FGF10. *Etv4-lacZ* or *Etv5-lacZ* (heterozygous) E11.5 kidneys were cultured for 3 days in normal medium (**a-b**) or with anti-GDNF blocking antibody (**c-f**), and with a control BSA-soaked bead (**a-d**) or FGF10-soaked bead (**e, f**), then stained for β-galactosidase. Expression of *Etv4-lacZ* or *Etv5-lacZ* is virtually extinguished by the anti-GDNF (c-d), but maintained in the vicinity of the FGF10 bead (e-f). The ability of FGF10 to upregulate *Etv4* and *Etv5*, even when GDNF signaling is blocked, indicates that it does not act by increasing GDNF expression.



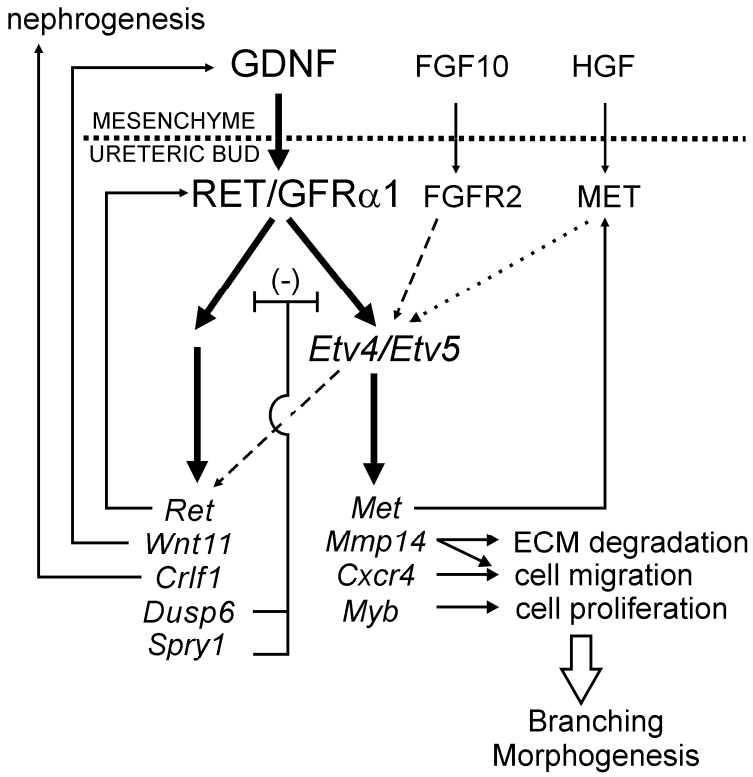
Supplementary Figure 2. Expression of *Etv4* and *Etv5* is not decreased by loss of *Fgf10*, or by loss of *Fgfr2* in the UB, but can be increased by excess FGF10. a-d, Expression of Etv4 and Etv5 mRNAs in *Fgf10-/-* kidneys (c,d) (E14.5) is similar to that in WT kidneys (a,b). **e-h**, Expression of Etv4 and Etv5 mRNAs in kidneys lacking *Fgfr2* in the UB (g,h) (E15.5) is similar to that in WT kidneys (e,f). Arrows, UB tips; arrowheads, nascent nephrons. **i-l,** Culture of E12.5 kidneys for 72 hrs with beads soaked in FGF10 (blue asterisks) upregulates expression of Etv4 and Etv5 in the UB (i,j), while FGF7 beads (black asterisks) or BSA control beads (red asterisks) do not (k,l). **m-n**, In control E14.5 lung cultures, both FGF7 and FGF10 beads induce enlargement of adjacent lung branches, confirming the activity of the two FGFs. Scale bars100 μM.



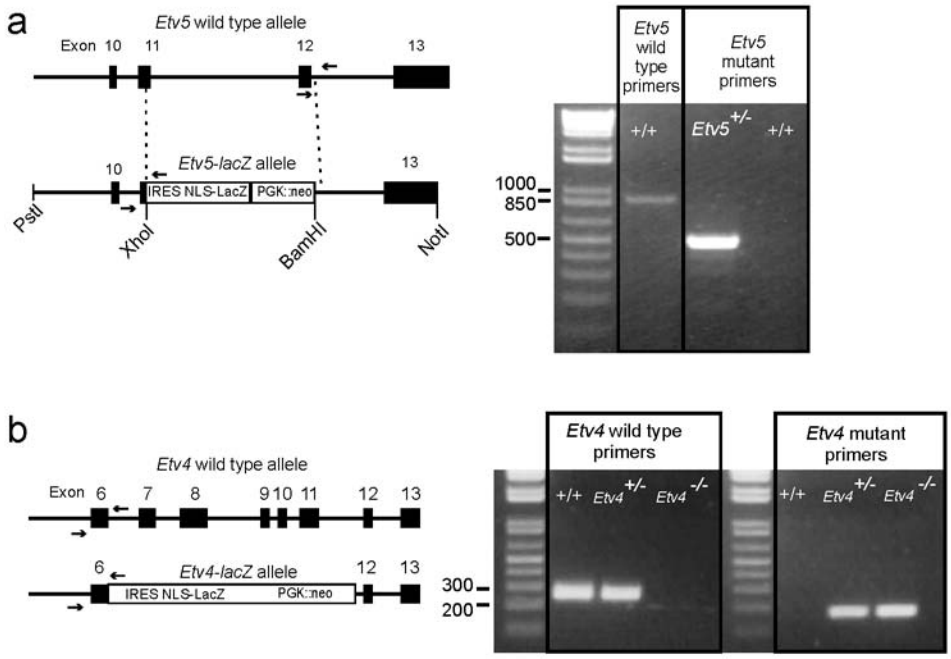
Supplementary Figure 3. Expression of *Etv4* and *Etv5* in the UB tips requires PI3K, but not Erk MAP kinase or PLC-γ signaling. a-d, expression of Etv4 and Etv5 mRNAs in UB tips of $Ret^{Y1015F/-}$ or control E14.5 kidneys. The Ret^{Y1015F} mutation fully blocks PLC-γ phosphorylation and causes abnormal UB branching, but has no effect on *Etv4* and *Etv5* expression. **e-h**, expression of Etv4 and Etv5 mRNAs in UB tips of $Ret^{Y1062F/Y1062F}$ or control E14.5 kidneys. The Ret^{Y1062F} mutation reduces, but does not eliminate both PI3K and Erk MAP kinase signaling, and causes reduced UB branching, but it has no effect on *Etv4* and *Etv5* expression (whole mount ISH). **i-p**, culture of WT E11.5 kidneys (carrying *Hoxb7/GFP*) with 50 μM PD98059 (specific inhibitor of Erk MAP kinase kinase) or 20 μM LY294002 (specific inhibitor of PI3K) causes moderately (PD98059) or severely (LY294002) reduced UB branching, consistent with previous observations (see text), thus confirming the efficacy of the inhibitors. **q-t**, expression of Etv4 and Etv5 shows little or no reduction in UB tips (arrows) of WT kidneys cultured with PD98059 for 48 hours, although there are fewer UB tips. **u-x,** culture of WT kidneys with LY294002 for 48hrs eliminates expression of Etv4 and Etv5.



Supplementary Figure 4. Normal expression of markers of metanephric mesenchyme, stroma and nascent nephrons in *Etv4*$^{-/-}$;*Etv5*$^{+/-}$ kidneys.
Gene expression was examined by ISH or (for Pax2) antibody staining. The markers label renal stroma (*Foxd1*), metanephric mesenchyme (*Gdnf, Wt1*, *Six1*, *Six2, Eya1*, *Pax2*), renal vesicles (*Pax2*, *Lim1, Jag1*), comma-and S-shaped shaped bodies (*Lim1, Jag1*), and podocytes (*Wt1, Lim1*). Although there are fewer UB tips and correspondingly fewer caps of MM and nephrons in the *Etv4*$^{-/-}$;*Etv5*$^{+/-}$ kidneys, the expression of all these genes occurs at similar levels to the wild type. Scale bars = 100 μM. Kidneys were at E13.5 (*Foxd1*), E14.5 (*Wt1*, Six2, *Jag1*, *Pax2*), E15.5 (*Gdnf*, *Six2*, *Lim1*), or E11.5 + 2 days culture (*Eya1*).



Supplementary Figure 5. Proposed role of *Etv4* and *Etv5* in a gene network controlling ureteric bud branching morphogenesis. GDNF from the mesenchyme signals to the UB via RET and GFRα1, thus regulating a set of downstream genes (bold arrows), including *Etv4* and *Etv5*. FGF10 signaling normally has a lesser role, if any, in regulating *Etv4/Etv5* expression in the UB (dashed arrow). Several of the genes downstream of *Ret* (e.g., *Met*, *Mmp14*, *Cxcr4*, *Myb*) are apparently regulated via *Etv4* and *Etv5*, as their expression is extinguished in *Etv4*$^{-/-}$*;Etv5*$^{+/-}$ kidneys. Other GDNF-induced genes (e.g., *Ret, Wnt11, Crlf1, Dusp6, Spry1*) are relatively insensitive to *Etv4/Etv5* levels (dashed arrow) and are likely regulated via other mechanisms. Collectively, these downstream genes compose part of a regulatory network that promotes UB branching morphogenesis, and other aspects of kidney development. In three positive feedback loops, Ret signaling positively regulates its own expression, *Wnt11* upregulates *Gdnf,* and *Met* encodes the receptor for HGF, which may also upregulate *Etv4/Etv5* (dotted arrow). *Spry1* and *Dusp6* are inhibitors of RTK signaling that participate in negative feedback loops. *Crlf1* may participate in nephrogenesis. *Mmp14*, *Cxcr4*, and *Myb* are likely to contribute to cellular processes important for UB morphogenesis (see Discussion).



Supplementary Figure 6. Construction of *Etv5-lacZ* allele, and genotyping of *Etv4-lacZ* and *Etv5-lacZ* alleles. a, schematic diagram of wild type *Etv5* and targeted *Etv5-lacZ* allele and PCR assays used for genotyping mice. Arrows indicate locations of PCR primers used for genotyping. One pair of primers amplifies a band of 833 bp from only the wild type *Etv5* allele, while a second primer pair amplifies a band of ~500 bp from the *Etv5-lacZ* (but not wild type) allele. **b**, schematic diagram of wild type *Etv4* and *Etv4-lacZ* alleles and PCR assays used for genotyping mice. One pair of primers amplifies a band of 247 bp from only the wild type *Etv4* allele, while a second primer pair amplifies a band of ~200 bp from the *Etv4-lacZ* (but not wild type *Etv4*) allele.

Supplementary Table 1 (following 2 pages). Changes in gene expression patterns in ureteric buds cultured +/- GDNF (Murine Genome Set U74Av2). Data are sorted for "fold change". Only genes with >2-fold change are listed. Full data are available at GEO.

Supplementary Table 1. Chip U74Av2

gene	fold change	lower bound of FC	upper bound of FC
Wnt11: wingless-related MMTV integration site 11	27.37	15.98	76.07
Cxcr4: chemokine (C-X-C motif) receptor 4	14.75	8.42	22.83
Crlf1: cytokine receptor-like factor 1	5.00	3.19	8.69
Arg2: arginase type II	4.24	3.62	5.02
Myb: myeloblastosis oncogene	3.86	2.11	12.52
Ret: ret proto-oncogene	3.72	2.1	6.26
Car2: carbonic anhydrase 2	3.52	2.17	5
Mtm1: X-linked myotubular myopathy gene 1	3.35	2.7	4.31
E2f8: E2F transcription factor 8	3.34	2.77	4.08
Dusp6: dual specificity phosphatase 6	3.32	1.84	16.18
Limch1: LIM and calponin homology domains 1	3.14	2.11	5.71
Ncaph: non-SMC condensin I complex, subunit H	3.04	2.21	4.79
Etv4: ets variant gene 4 (E1A enhancer binding protein, E1AF)	3.03	2.33	3.91
Kit: kit oncogene	3.02	2.13	4.2
C330027C09Rik: RIKEN cDNA C330027C09 gene	2.98	2.06	4.87
Ect2: ect2 oncogene	2.96	2.57	3.48
Spred2: sprouty-related, EVH1 domain containing 2	2.96	1.92	6.17
Ncaph: non-SMC condensin I complex, subunit H	2.94	2.1	4.56
2810417H13Rik: RIKEN cDNA 2810417H13 gene	2.88	2.18	3.94
Snrpd3: small nuclear ribonucleoprotein D3	2.87	1.88	4.4
Scd1: stearoyl-Coenzyme A desaturase 1	2.86	1.82	4.86
Mycn: v-myc myelocytomatosis viral related oncogene, neuroblasto	2.83	2.09	3.63
Mki67: antigen identified by monoclonal antibody Ki 67	2.83	2.02	4.53
Cdca8: cell division cycle associated 8	2.72	1.98	4.12
Ccnd1: cyclin D1	2.67	1.62	6.81
2700099C18Rik: RIKEN cDNA 2700099C18 gene	2.59	1.52	8.73
LOC14210: hypothetical LOC14210	2.59	1.47	4.15
Bub1: budding uninhibited by benzimidazoles 1 homolog (S. cerevisi	2.58	1.47	6.19
Gmnn: geminin	2.56	1.97	3.61
Ctnnd2 /// LOC100045979: catenin (cadherin associated protein), d	2.54	2.03	3.15
Rrm2: ribonucleotide reductase M2	2.53	1.77	4.07
Ccnb1: cyclin B1	2.47	1.73	4.06
Dna2: DNA replication helicase 2 homolog (yeast)	2.45	1.62	4.21
Kif20a: kinesin family member 20A	2.44	2.04	2.99
Cenpk: centromere protein K	2.42	1.86	3.39
Nusap1: nucleolar and spindle associated protein 1	2.42	1.91	3.27
Topbp1: topoisomerase (DNA) II binding protein 1	2.40	1.65	3.58
Smc4: structural maintenance of chromosomes 4	2.40	2.00	2.93
Kif11: kinesin family member 11	2.39	1.85	3.32
Mest: mesoderm specific transcript	2.38	1.66	3.99
Ccnb2: cyclin B2	2.37	1.77	3.41
Uhrf1: ubiquitin-like, containing PHD and RING finger domains, 1	2.37	1.78	3.48
Top2a: topoisomerase (DNA) II alpha	2.37	1.51	5.12
LOC100045677: similar to DNA replication licensing factor MCM3 (D	2.37	1.6	4.26
Hmgb2 /// OTTMUSG00000011058: high mobility group box 2 /// pı	2.33	1.35	6.4
Kif20a: kinesin family member 20A	2.33	1.37	4.39
Usp1: ubiquitin specific peptdiase 1	2.31	1.51	4.7
Smc2: structural maintenance of chromosomes 2	2.29	1.75	3.16

Supplementary Table 1. Chip U74Av2

Plk4: polo-like kinase 4 (Drosophila)	2.29	1.7	3.29
Cadm1: cell adhesion molecule 1	2.28	2.07	2.53
Kif4: kinesin family member 4	2.28	1.79	3.12
Ahcy: S-adenosylhomocysteine hydrolase	2.26	1.63	3.64
Rad51: RAD51 homolog (S. cerevisiae)	2.25	1.71	3.12
Cdc6: cell division cycle 6 homolog (S. cerevisiae)	2.24	1.75	2.88
Cdc7: cell division cycle 7 (S. cerevisiae)	2.23	1.87	2.7
Kif2c /// LOC631653: kinesin family member 2C /// similar to Kinesi	2.23	1.7	3.19
Plk1: polo-like kinase 1 (Drosophila)	2.22	1.36	4.04
Gnl3: guanine nucleotide binding protein-like 3 (nucleolar)	2.21	1.53	3.19
Cks2 /// LOC100044764 /// LOC100046104 /// OTTMUSG00000022(	2.21	1.49	3.98
Rrs1: RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)	2.21	1.4	4.39
Trip13: thyroid hormone receptor interactor 13	2.21	1.55	3.65
Cdc25c: cell division cycle 25 homolog C (S. pombe)	2.2	1.73	2.86
Tacc3: transforming, acidic coiled-coil containing protein 3	2.19	1.45	4.08
Prim1: DNA primase, p49 subunit	2.19	1.61	3.37
Gldc: glycine decarboxylase	2.18	1.46	3.14
Pola1: polymerase (DNA directed), alpha 1	2.17	1.68	3.04
Birc5: baculoviral IAP repeat-containing 5	2.15	1.7	2.83
Shmt1: serine hydroxymethyltransferase 1 (soluble)	2.15	1.46	3.16
Narg1: NMDA receptor-regulated gene 1	2.15	1.48	3.64
ENSMUSG00000045455 /// Timm8a1: predicted gene, ENSMUSG00	2.14	1.63	3.08
Nfib: nuclear factor I/B	2.14	1.36	3.81
Mad2l1: MAD2 mitotic arrest deficient-like 1 (yeast)	2.13	1.8	2.56
Crip2: cysteine rich protein 2	2.13	1.6	2.91
Dusp9: dual specificity phosphatase 9	2.13	1.51	3.52
Limch1: LIM and calponin homology domains 1	2.13	1.89	2.41
Anp32e: acidic (leucine-rich) nuclear phosphoprotein 32 family, mer	2.12	1.39	4.31
Tcf7: transcription factor 7, T-cell specific	2.11	1.38	3.44
AU020206: expressed sequence AU020206	2.11	1.73	2.68
Ilf2: interleukin enhancer binding factor 2	2.1	1.57	2.91
Clic4: chloride intracellular channel 4 (mitochondrial)	2.1	1.73	2.56
Uck2: uridine-cytidine kinase 2	2.08	1.22	4.32
Ahcy: S-adenosylhomocysteine hydrolase	2.07	1.78	2.38
Rpa2: replication protein A2	2.06	1.51	3.07
Brca1: breast cancer 1	2.06	1.59	2.81
LOC630539 /// Trim59: similar to mouse RING finger 1 /// tripartite	2.05	1.29	3.74
Cdca7l: cell division cycle associated 7 like	2.05	1.66	2.49
Fubp1: far upstream element (FUSE) binding protein 1	2.04	1.58	2.53
Prc1: protein regulator of cytokinesis 1	2.04	1.38	3.38
Hells: helicase, lymphoid specific	2.04	1.43	3.03
Fignl1: fidgetin-like 1	2.04	1.55	2.84
Dck: deoxycytidine kinase	2.03	1.66	2.5
Aurkb: aurora kinase B	2.03	1.62	2.7
Prkrir: protein-kinase, interferon-inducible double stranded RNA de	2.03	1.68	2.48
Aurka: aurora kinase A	2	1.26	3.61
Wnt4: wingless-related MMTV integration site 4	-2.19	-1.25	-3.25
Znrf2: zinc and ring finger 2	-2.38	-1.95	-2.91
5728807_RC	-2.4	-1.85	-3.4

Supplementary Table 2 (following 2 pages). Changes in gene expression patterns in ureteric buds cultured +/- GDNF (Murine Genome Set 430A). Data are sorted for "fold change". Only genes with >2-fold change are listed. Full data are available at GEO.

Supplementary Table 2. Chip 430A

gene	fold change	lower bound of FC	upper bound of FC
Etv5: ets variant gene 5	5.52	3.06	12.37
Cxcr4: chemokine (C-X-C motif) receptor 4	5.17	3.87	6.95
Crlf1: cytokine receptor-like factor 1	4.38	3.14	6.76
Myb: myeloblastosis oncogene	3.27	2.11	6.21
LOC100046643 /// Spry1: similar to sprouty 1 /// sprouty homo	3.26	2.51	4.34
Ccnd1: cyclin D1	3.16	2.16	5.02
Ncapg: on-SMC condensin I complex, subunit G	2.95	2.47	3.63
Ret: ret proto-oncogene	2.88	1.83	4.49
Mtm1: X-linked myotubular myopathy gene 1	2.87	2.31	3.54
Etv4: ets variant gene 4 (E1A enhancer binding protein, E1AF)	2.85	1.89	3.95
Ccnd1: cyclin D1	2.84	2.36	3.54
Arg2: arginase type II	2.8	2.29	3.42
Hist1h2ab /// Hist1h2ac /// Hist1h2ad /// Hist1h2ae /// Hist1h2	2.72	2.34	3.18
Cdc6: cell division cycle 6 homolog (S. cerevisiae)	2.7	1.64	3.87
Arg2: arginase type II	2.69	2.23	3.25
Fabp5: fatty acid binding protein 5, epidermal	2.66	1.83	4.24
Ccnb1 /// EG434175 /// EG667005: cyclin B1 /// predicted gene	2.66	1.77	4.62
Psph: phosphoserine phosphatase	2.62	1.86	4.12
Mcm6: minichromosome maintenance deficient 6 (MIS5 homol	2.62	1.81	4.67
Ccnd1: cyclin D1	2.58	1.81	4.28
Mtm1: X-linked myotubular myopathy gene 1	2.55	1.94	3.52
Dusp6: dual specificity phosphatase 6	2.53	1.59	5.75
Ung: uracil DNA glycosylase	2.51	2.07	3.15
EG620603 /// Fabp5: predicted gene, EG620603 /// fatty acid b	2.5	2.05	3.18
Smc4: structural maintenance of chromosomes 4	2.5	1.6	5.06
Vldlr: very low density lipoprotein receptor	2.46	1.96	3.12
Etv5: ets variant gene 5	2.46	1.62	3.82
Asns: asparagine synthetase	2.45	1.93	3.32
Ube2c: ubiquitin-conjugating enzyme E2C	2.44	1.77	3.7
Ccnb2: cyclin B2	2.43	2.04	2.91
2810433K01Rik: RIKEN cDNA 2810433K01 gene	2.35	1.69	3.76
Lmnb1: lamin B1	2.34	1.67	3.45
Hells: helicase, lymphoid specific	2.33	1.88	3.01
Ncaph: non-SMC condensin I complex, subunit H	2.28	1.86	2.91
Mest: mesoderm specific transcript	2.25	1.48	3.31
Dck: deoxycytidine kinase	2.24	1.68	2.92
Uhrf1: ubiquitin-like, containing PHD and RING finger domains,	2.23	1.77	2.98
Ect2: ect2 oncogene	2.23	2	2.53
Brca1: breast cancer 1	2.23	1.74	3
Smc2: structural maintenance of chromosomes 2	2.22	1.75	3
Rrm2: ribonucleotide reductase M2	2.21	1.73	2.9
Bub1: budding uninhibited by benzimidazoles 1 homolog (S. cer	2.21	1.9	2.6
Fbxl10: F-box and leucine-rich repeat protein 10	2.21	1.73	2.86
Incenp: inner centromere protein	2.21	1.69	2.98
Ctnnd2 /// LOC100045979: catenin (cadherin associated protei	2.2	1.91	2.57

Supplementary Table 2. Chip 430A

Spred2: sprouty-related, EVH1 domain containing 2	2.2	1.87	2.61
Rfc4: replication factor C (activator 1) 4	2.2	1.61	3.42
Cep55: centrosomal protein 55	2.19	1.9	2.57
Rad51: RAD51 homolog (S. cerevisiae)	2.18	1.72	2.87
Prpf40a: PRP40 pre-mRNA processing factor 40 homolog A (yea	2.18	1.45	3.18
Snrpd3: small nuclear ribonucleoprotein D3	2.16	1.43	2.94
Gpsm2: G-protein signalling modulator 2 (AGS3-like, C. elegans)	2.15	1.87	2.48
Sostdc1: sclerostin domain containing 1	2.14	1.63	2.7
Dut: deoxyuridine triphosphatase	2.14	1.84	2.5
2810417H13Rik: RIKEN cDNA 2810417H13 gene	2.13	1.8	2.56
Cadm1: cell adhesion molecule 1	2.12	1.84	2.44
Wnt11: wingless-related MMTV integration site 11	2.12	1.47	3.02
Ccnb1: cyclin B1	2.11	1.75	2.59
Shcbp1: Shc SH2-domain binding protein 1	2.11	1.7	2.72
Mki67: antigen identified by monoclonal antibody Ki 67	2.11	1.83	2.49
Spry2: sprouty homolog 2 (Drosophila)	2.11	1.69	2.71
Trib2: tribbles homolog 2 (Drosophila)	2.1	1.36	3
Cdc2a: cell division cycle 2 homolog A (S. pombe)	2.09	1.57	2.82
Ccna2: cyclin A2	2.09	1.79	2.49
Kif22: kinesin family member 22	2.09	1.45	3.65
LOC14210: hypothetical LOC14210	2.07	1.68	2.62
Racgap1: Rac GTPase-activating protein 1	2.07	1.81	2.36
Top2a: topoisomerase (DNA) II alpha	2.07	1.55	2.99
Cth: cystathionase (cystathionine gamma-lyase)	2.07	1.57	3
Ctps: cytidine 5'-triphosphate synthase	2.06	1.7	2.6
Gpt2: glutamic pyruvate transaminase (alanine aminotransferas	2.06	1.57	2.88
100039943 /// 100040234 /// 100041386 /// 100041667 /// 10	2.05	1.59	2.87
Exo1: exonuclease 1	2.05	1.68	2.55
Fignl1: fidgetin-like 1	2.05	1.75	2.42
Nol5: nucleolar protein 5	2.05	1.75	2.45
Plk4: polo-like kinase 4 (Drosophila)	2.05	1.9	2.22
Kif20a: kinesin family member 20A	2.04	1.46	2.98
Birc5: baculoviral IAP repeat-containing 5	2.04	1.75	2.44
Idi1: isopentenyl-diphosphate delta isomerase	2.04	1.67	2.61
Trip13: thyroid hormone receptor interactor 13	2.04	1.85	2.28
Rrm2: ribonucleotide reductase M2	2.04	1.78	2.37
LOC100045677 /// Mcm3: similar to DNA replication licensing fa	2.04	1.52	2.89
Vldlr: very low density lipoprotein receptor	2.03	1.73	2.38
Aldh1a1: aldehyde dehydrogenase family 1, subfamily A1	2.02	1.37	2.79
Ccne2: cyclin E2	2.01	1.64	2.56
LOC100045677 /// Mcm3: similar to DNA replication licensing fa	2.01	1.33	3.4
Slc40a1: solute carrier family 40 (iron-regulated transporter), m	-2.09	-1.54	-2.89
H2-D4: histocompatibility 2, D region locus 4	-2.1	-1.67	-2.7
Pth1r: parathyroid hormone 1 receptor	-2.11	-1.49	-2.88
Hspb1: heat shock protein 1	-2.23	-2.03	-2.46
Hspb1: heat shock protein 1	-2.23	-2	-2.51

a) Etv4-lacZ mutants

Etv4	*Etv5*	N	normal	hypoplastic kidney	kidney absent	ureter absent
+/+	+/+	43	100%	0%	0%	0%
+/-	+/+	90	96%	4%	0%	0%
-/-	+/+	75	89%	9%	2%	0%

b) Etv4-lacZ; Etv5-lacZ compound mutants

Etv4	*Etv5*	N	normal	hypoplastic kidney	kidney absent	ureter absent
+/+	+/-	27	100%	0%	0%	0%
+/-	+/-	46	76%	15%	8%	5%
-/-	**+/-**	**37**	**8%**	**52%**	**41%**	**22%**

c) Etv4-lacZ ; Etv5^{M} compound mutants

Etv4	*Etv5*	N	normal	hypoplastic kidney	kidney absent	ureter absent
+/+	+/M	26	100%	0%	0%	0%
+/+	M/M	9	100%	0%	0%	0%
+/-	+/M	50	100%	0%	0%	0%
-/-	+/M	35	71%	7%	21%	0%
+/-	M/M	25	58%	18%	24%	0%
-/-	**M/M**	**7**	**0%**	***** 7%**	**93%**	**29%**

Supplementary Table 3. Frequency of renal and ureteric defects in single and compound *Etv4/Etv5* mutant newborn mice with two different *Etv5* knockout alleles. **a**, wild types and *Etv4-lacZ* mutants. **b**, *Etv5-lacZ* mutants and *Pea-lacZ;Etv5-lacZ* compound mutants. No *Etv5-lacZ* homozygotes were obtained, as this allele is recessive lethal at E8.5. **c**, *Etv5*^{M} mutants and *Etv4-lacZ;Etv5*^{M} compound mutants. ***, extreme hypodysplasia – see Fig. 4 i, k. N = number of mice analyzed. The two most severely affected genotypes are shown in bold.

Etv4-lacZ; Etv5-lacZ compound mutants

Etv4	*Etv5*	N	normal branching	reduced branching	no branching	UB absent
controls		68	93%	7%	0%	1%
-/-	+/+	26	79%	13%	6%	2%
+/-	+/-	25	78%	16%	2%	4%
-/-	+/-	29	26%	19%	40%	16%

Supplementary Table 4. Frequency of defects in the *in vivo* outgrowth and early branching of the ureteric bud in *Etv4-lacZ;Etv5-lacZ* single and compound mutants. Embryos were evaluated at E11.5 – E12.5. Controls include wild types, *Etv4*$^{+/-}$ heterozygotes and *Etv5*$^{+/-}$ heterozygotes. N= number of embryos analyzed.

allele	primer 1	primer 2	product
***Etv4* WT**	5’ CGGTCTTCAGAAAGCAGAGG 3'	5’ GAGCCTTCGACTCCTGACAC 3'	247 bp
Etv4-lacZ	5’ GAAGCCACCACTCCCCTAC 3’	5’ CCAAAAGACGGCAATATGGT 3’	~ 200 bp
***Etv5* WT**	5' ATTAGCAGCTCACTAGCATGTTCC 3'	5' CGTTGAGCCAGCCACTTCTTC 3'	833 bp
Etv5-lacZ	5’ AAGCACAGCCTCTGTTGAGC 3’	5’ ACACCGGCCTTATTCCAAG 3’	~ 500 bp

Supplementary Table 5. PCR primers used for genotyping Etv4-lacZ and Etv5-laz mice.